COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, **Jennifer Ruth Gamble** of the Hanson Centre for Cancer Research, in the Institute of Medical and Veterinary Science, Frome Rd, Adelaide, South Australia, 5000 declare as follows:

1. Scientific And Professional History.

1.1 I am the Co-Head of the vascular biology unit at the Hanson Centre for Cancer Research. I am also an Associate Professor with the Department of Medicine at the University of Adelaide, a position that I have held since 1998. I am a Research Hospital Scientist with the Institute of Medical and Veterinary Science in Adelaide and a foundation member and co-head of the vascular biology unit of the Hanson Centre for Cancer Research, which was established in 1989.

1.2 I received my Bachelor of Applied Science degree from the Royal Melbourne Institute of Technology, Victoria in 1978. I went on to receive my Masters of Science degree from The Walter and Eliza Hall Institute of Medical Research at the University of Melbourne in 1986. Subsequently

I received my Doctorate of Philosophy from University of Adelaide in 1994.

1.3 My research studies over the last fifteen years have been in the general field of immunology and vascular biology. In particular, I have been studying the cells that form the lining of blood vessels and lymphatic vessels, known as the endothelium. These endothelial cells are involved in the processes of inflammation and new blood vessel growth, the latter process being termed angiogenesis. A specific area of interest has been the response of vascular endothelial cells to a group of molecules, termed growth factors, which affect the growth and behaviour of cells. In my research I have worked with many different growth factors including colony stimulating factors, interleukins and vascular endothelial growth factors ("VEGFs").

1.4 The study of angiogenesis is of enormous clinical significance because the growth of new blood vessels play an important role in biological processes such as wound healing and tumour development.

1.5 Nearly all cells in a vertebrate such as a mammal are located within 50μm of a capillary so as to receive oxygen and other nutrients from the circulatory system. Cells receiving insufficient oxygen usually release signalling proteins (eg growth factors) that stimulate the growth of blood vessels towards those cells. Thus, after wounding, a burst of capillary growth is stimulated in the neighbourhood of the damaged tissue to provide a new network of blood vessels to supply the healing tissue and to replace those blood vessels damaged by the injury. It is thought that the lack of oxygen to damaged tissue causes the release of growth factors by those cells, which triggers the angiogenic process. However, the process of angiogenesis takes time and can be insufficient or ineffective in certain situations. For example, ischemic damage takes place in catastrophic events such as heart attacks, strokes and angiogenesis is insufficient in other ischemic conditions such as in critical limb ischemia. Further, after surgery, the body's response may not be sufficient to promote satisfactory healing. Consequently, there is great interest in the development of a means to stimulate the body's natural angiogenic processes.

1.6 Tumours, like any other body tissue, also need a suitable blood supply. However, the growth of a solid tumour is often limited by its blood supply.

Consequently, a critical step in tumour progression is induction of capillary growth into the tumour mass. To do this, the tumour cells have to provide signals to existing capillaries to branch out and grow new vessels into the tumour. Unless a tumour is able to do this, it will be unable to grow more than a few millimetres in diameter. Tumours elicit new vessel growth by the nature of their ischemic environment and by secreting the same angiogenic growth factors as normal cells. There is, therefore, considerable clinical interest in elucidating the normal angiogenic process so that methods can be devised for inhibiting tumour growth by blocking the process.

1.7 Since about 1991, the vast majority of the work that I have done has involved cell biology and biochemical studies of endothelial cell function focussing on the regulatory effects of growth factors. I have authored or co-authored over eighty-five scientific papers in refereed publications. My research team and I have also been searching for novel factors involved in the process of angiogenesis. Since 1993 we have isolated a number of possible candidate molecules. The isolation of angiogenically important molecules has now developed into a major area of interest for my laboratory.

1.8 Examples of my research are described in many of my publications listed in my *curriculum vitae*. Now produced and shown to me marked "**Annexure JRG-1**" is a true copy of my *curriculum vitae*, which identifies the publications of which I have been an author or co-author.

1.9 Thus, as a Research Scientist, and subsequently co-head of the vascular biology unit, at the Hanson Centre for Cancer Research, I have conducted and directed an extensive amount of research in the field of angiogenesis and endothelial cell biology.

1.10 Now produced and shown to me marked "**Annexure JRG-2**" is a summary of the research that I have conducted over the last fifteen years.

1.11 Thus, I have extensive knowledge of the field of angiogenesis and endothelial cell biology, and its development in Australia over the last fifteen years. As such, I am familiar with the background knowledge, experience and technical abilities of researchers in my field, especially in Australia over the last fif[illegible] years through my research, writing, supervisory responsibilities and referee duties for various international

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journals. I have been required to develop and maintain an extensive knowledge of the Australian and international scientific literature for a diverse range of scientific fields including molecular biology, biochemistry, cell biology, and angiogenesis etc.

1.12 In the following sections I refer to various scientific publications and patent specifications. Unless otherwise identified, I have not enclosed copies of these documents with this statutory declaration since the patent attorneys representing Human Genome Sciences Inc ("HGS") have informed me that copies of these documents will be filed by separate means.

2 My Instructions

2.1 I am informed by the patent attorneys representing HGS that this proceeding concerns an opposition by Ludwig Institute for Cancer Research to Australian Patent Application Au-B-696764 (73941/94) in the name of HGS and entitled "Vascular Endothelial Growth Factor 2" ("the patent specification"), which has an earliest date of filing of 8 March 1994 ("March 1994"). I have been asked to provide my comments and opinions on the patent specification for use in these proceedings. My opinions concerning the content (information) in the patent specification are contained in this statutory declaration.

2.2 The patent attorneys for HGS have provided me with a copy of a document entitled "*Guidelines for Expert Witnesses in Proceedings in the Federal Court of Australia.*"

2.3 The patent attorneys for HGS have provided me with copies of various documents. Now produced and shown to me marked "**Annexure JRG-3**" is a list of those documents. I have been asked to review those documents and to provide my comments thereon. My decision not to address each passage in each of the statutory declarations that I have reviewed in a consecutive order should not be viewed or taken as an admission on my part of acceptance of any text that I do not comment on. Rather, I have found much of the evidence to be repetitive and have structured my comments in a manner to avoid excessive repetition.

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3 Summary of The Patent Specification

3.1 The invention disclosed in the patent specification relates generally to vascular endothelial growth factor 2 ("VEGF-2"). Today, VEGF-2 is identified by the nomenclature VEGF-C. When I read VEGF-2 in the patent specification I understand it to mean VEGF-C. For the sake of consistency with the patent specification, however, I shall use the term VEGF-2 in this statutory declaration.

3.2 I would classify the general field of the invention described in the patent specification as the identification of a novel growth factor that is active in the process of endothelial cell growth and differentiation. In this statutory declaration I refer to that general field as the "field of endothelial cell growth and differentiation". Endothelial growth factors are particularly active in the process known as angiogenesis, i.e. the process leading to the formation of new blood vessels from pre-existing vessels.

3.3 I note that the specification is principally directed to a disclosure of a human VEGF-2 gene sequence and polypeptide sequences and its uses. The disclosure in the patent specification does not, however, end with that information since the specification also discloses recombinant techniques and procedures for producing polypeptides from VEGF-2 gene sequences. More specific detail concerning the information in the patent specification is provided in section 6 below.

4 The Field of Endothelial Cell Growth and Differentiation, including Angiogenesis in March 1994

4.1 I have been asked by the patent attorneys for HGS to comment on the state of knowledge of researchers working in the field of endothelial cell growth and differentiation, which includes angiogenesis, leading up to the development of the invention described in the patent specification, as I understood it to be at March 1994. In providing these comments I rely not only upon my research and experience, but also substantially rely on discussions I had prior to March 1994 with others in my field and the results of research and developments published by others in the field of vascular biology generally around the world in scientific journals. I assess published work by others for myself and if I consider it to be sound and relevant. use it as a basis of knowledge and assistance in my own research.

4.2 In providing my comments I have to the best of my ability endeavoured to be mindful not to take into account, unless otherwise stated, any information that I have subsequently obtained after March 1994.

Standard of Knowledge in Australia

4.3 In my opinion the standard of knowledge in the field of endothelial cell growth and differentiation, including angiogenesis, in Australia was equivalent to that anywhere else in the world as of March 1994. A number of Australian groups were actively working in the field in and prior to March 1994. By way of example, I consider that my work on the regulation of endothelial adhesion, in developing an improved gel-type assay capable of assaying for the biological activity of endothelial growth factors and my work on integrins, are some examples of work that was significant for this field.

4.4 Technology and scientific developments in the field of endothelial cell growth and differentiation both outside and within Australia are now, and were prior to March 1994, transmitted rapidly throughout the world through medical and scientific journals and publications, through telephone conversations with colleagues overseas, through computer links with overseas databases, from conferences held in Australia at which overseas researchers and practitioners made presentations, and from Australian researchers and practitioners returning from overseas conferences.

4.5 One of the ways in which I and others identified scientific publications in a field of interest was by the use of the *Medline* electronic database maintained by the National Library of Medicine in the United States of America and containing an index to the world's biomedical literature. Access to *Medline* in an on-line format has been available in Australia since before March 1994. The *Medline* database enables searches to be conducted of the medical and scientific literature for papers relating to particular topics of interest. I, and others with whom I have worked, have regularly used the *Medline* database as a research tool, as a matter of routine, to locate articles on particular topics since before March 1994.

4.6 In addition, many Australian researchers had done post-doctoral studies in overseas laboratories in institutions, as I had, and had kept connections with those laboratories and institutions so that a lot of

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knowledge is and was transmitted by personal communication, often before publication. Some overseas researchers and practitioners also came to work in Australian laboratories and institutions.

4.7 Thus to summarise, in my opinion, the general state of knowledge in the area of endothelial cell growth and differentiation, including angiogenesis, in Australia in March 1994 would not have been greatly different from that in other developed and industrialised countries such as the United States of America and many countries in Europe. Based on my experience, the general level of skill of persons working in laboratories and research institutions in Australia would have been similar to that of persons working in overseas laboratories and institutions.

Background Knowledge in the field in March 1994

4.8 By March 1994 a lot of information was known about endothelial cell growth and differentiation, including angiogenesis. By way of example of the information that was known by my colleagues and me, I refer to two review articles that my colleagues and I wrote, in 1994 and in 1995 that described the general state of this field. Those articles were:

(a) Cockerill, Gamble, Vadas (1994) "Angiogenesis: models and model vectors". In: International Reviews of Cytology. A Survey of Cell Biology 159: 113-160; and

(b) Litwin, Gamble, Vadas (1995) "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129.

4.9 These review articles are identified as publications 58 and 59 respectively in my *Curriculum Vitae*. Although document (b) was published in 1995 the majority of the research reported concerns data published prior to 1994 and or that we were aware of prior to 1994.

4.10 Endothelial cells form the inner lining of the blood and lymphatic vessels of vascular systems. Endothelial cells play a critical role in physiological and pathological processes, including inflammatory responses, wound healing and the generation of new vessels.

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4.11 The generation of vessels of the vascular system is termed vasculogenesis or angiogenesis. Vasculogenesis is mainly used to describe the *de novo* generation of vessels occurring during embryogenesis.

4.12 The term angiogenesis is routinely used to summarise a myriad of different cellular events that occur after vasculogenesis that lead to the development of new blood vessels through sprouting from pre-existing vessels. This process involves the migration and proliferation of endothelial cells from pre-existing vessels. Angiogenesis is not limited to the embryonic period of development but also occurs in adults where the formation of vessels is required and is of particular significance in wound healing, maturation of ovarian follicles and tumour development.

4.13 Angiogenesis is known to be controlled by the release of growth factors from neighbouring tissues. By 1994, many different growth factors, had been shown to exert effects on the growth, differentiation and behaviour of cells of the vasculature including endothelial cells, smooth muscle cell and fibroblasts and were, therefore, expected to participate in angiogenic processes in one way or another. Examples of such growth factors included vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and placenta-derived growth factor (PIGF).

Endothelial Growth Factors and related proteins – PDGF & VEGF

5.1 As discussed above, one of my primary research interests prior to and after 1994 was the role of growth factors in regulating endothelial cell function. Indeed, in 1995, my colleagues and I co-wrote a review article that summarised the role of various growth factors in endothelial cell function (Litwin, Gamble, Vadas, 1995, "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129). I refer to that review as a sample of the knowledge and information that was known by 1994 about these growth factors, including VEGF and PDGF.

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(a) **PDGF**

5.2 Platelet-derived growth factor (PDGF) was identified in 1974 in human platelets as a potent growth factor for smooth muscle cells, fibroblasts and glial cells. Subsequent research established it to be a dimer. Cloning and sequencing of the two chains revealed that they are similar to each other and have been called PDGFα and β.

5.3 Both PDGFα and β possess N-terminal hydrophobic signal sequences that can direct secretion of the protein from the cells. Further, both forms are proteolytically processed to form the mature protein. PDGFα and β both contain the eight cysteine residues and 14 amino acid signature motif characteristic of all members of the PDGF/VEGF family.

5.4 Prior to 1994, PDGF was shown to stimulate the growth of connective tissue cells including smooth muscle cells. In addition to inducing cell replication, PDGF was also shown to elicit a number of intracellular signals related to mitogenesis, to be chemotactic, to act as a vasoconstrictor, to activate leukocytes, and to modulate extracellular matrix turnover. PDGF had also been shown to be involved in the regulation of cell growth and chemotaxis during embryonic development.

5.5 Two different types of receptor for PDGF have been identified, the alpha and beta receptors. They are both receptor tyrosine kinases.

(b) **VEGF**

5.6 Vascular endothelial cell growth factor (VEGF), was isolated in 1987 from foetal bovine retina and was shown to be highly specific for endothelial cells, by contrast to other known growth factors such as PDGF which affect a range of cell types.

5.7 In 1989 cDNA clones for both human and bovine VEGF were obtained. Analysis of the primary amino acid sequence of VEGF revealed some homology to the α and β chains of PDGF (18 to 20%). In particular, VEGF was shown to contain the same eight conserved cysteine residues that are also present in PDGF. VEGF also contains the 14 amino acid signature motif characteristic of all PDGF/VEGF family members.

5.8 VEGF was known to comprise a hydrophobic N-terminal signal sequence that directs secretion of the protein. VEGF, like PDGF, is proteolytically

processed to form the mature protein. Further, all forms of VEGF were known to exist as dimers, joined by disulphide bonds. However, unlike PDGF, the polypeptide making up the dimers of VEGF are identical.

5.9 The secreted forms of VEGF were demonstrated to promote the growth of vascular endothelial cells but not others, which contrasted to that seen for PDGF.

5.10 VEGF has been shown to increase blood vessel permeability, stimulate proliferation in vascular endothelial cells and promote angiogenesis. VEGF has also been shown to be expressed at high levels in a number of tumours and to confer Chinese Hamster Ovary (CHO) cells with the ability to form tumours in nude mice. These observations provided evidence for a role for VEGF in promoting the growth of new blood vessels during tumour development: the ability of solid tumours to stimulate the growth of new blood vessels is an essential part of their development.

5.11 In 1991, a receptor for VEGF was characterized, the Flt-1 receptor (also known as VEGFR-1). A further VEGF receptor, the KDR/Flk-1 receptor (also known as VEGFR-2) was identified in 1993. Both receptors are members of the receptor tyrosine kinase superfamily. Binding of VEGF to the receptor results in receptor phosphorylation.

(c) PlGF

5.12 Placenta growth factor (PlGF) was identified in 1991 from a placenta cDNA library. It is a 149 amino acid protein comprising a N-terminal hydrophobic signal sequence and proteolytic cleavage sites. Computer analysis revealed 53% identity to VEGF over the conserved PDGF-like region that comprises eight cysteine residues and the 14 amino acid signature motif characteristic of all members of the PDGF/VEGF family. Expression of the cDNA encoding PlGF resulted in a secreted protein in the form of a dimer of two identical chains. The recombinant protein was also shown to be capable of stimulating the growth of endothelial cells but not NIH3T3 fibroblast cells. This finding indicated that recombinantly produced PlGF protein had the same specificity for endothelial cells as VEGF.

5.13 Thus, PDGFα, PDGFβ, VEGF and PIGF are all secreted growth factors. Each of these proteins contains an N-terminal hydrophobic signal sequence and is proteolytically processed into a mature form. They all form dimers, either with themselves or with related proteins. Each of these proteins contains eight cysteine residues and the 14 amino acid signature motif characteristic of the PDGF/VEGF growth family, and they are all involved in angiogenesis.

Angiogenic Assays

5.14 The preceding paragraphs provide a summary of the known (i.e. by 1994) properties and biological activities of the PDGF/VEGF family of growth factors involved in angiogenesis. These properties and activities were determined using a number of bioassays, routinely used prior to 1994. These assays allowed my research team and others not only to distinguish between the various growth factors but also to explore further the biological properties of any growth factor of interest. For example, many of the bioassays allow direct determination of mitogenic and angiogenic activities and permit repeated, long-term quantitation of mitogenesis and angiogenesis as well as physiological characterization of angiogenic vessels. Some of the assays that were routinely available and used by my research team and others in the field, before 1994, are discussed in more detail below.

5.15 In 1994, common *in vitro* and *in vivo* assays available for measuring endothelial cell associated activity included:

(a) *In vitro* angiogenic assays

- i. Proliferation assay
- ii. Three Dimensional Gel assay
    1. Type I Collagen gels
    2. Matrigel
    3. Fibrin gels
- iii. Wound type assay
- iv. Permeability assay
- v. Aortic Ring model

(b) *In vivo* angiogenic assays

- i. Chicken Chorioallantoic Membrane assay

ii. Corneal Neovascularisation assay
iii. Pouch assay

5.16 Rather than provide an expansive discussion on each of the available assays in this Statutory Declaration I refer to Litwin, Gamble, Vadas, 1995, "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129, which my colleagues and I wrote in 1994, which provides details of a number of the assays listed above.

5.17 Prior to 1994 my laboratory staff and I routinely used three different assays to measure aspects of angiogenesis *in vitro*. They were the cell proliferation assay, three dimensional gel assay (which I improved in my laboratory, see below) and a wound assay. Many research teams around the world also routinely used these assays. Using these assays my team and I examined the angiogenic activity of a number of growth factors including endothelial growth factors such as VEGF.

5.18 Between 1992 and 1993 I also developed a high throughput *in vitro* angiogenic assay capable of assessing large numbers of angiogenic molecules. That assay was reported in Gamble J.R. *et al.*, 1993, Journal of Cell Biology 121: 931-934. Journal of Cell Biology is one of the standard journals in my field of research and one that my colleagues and I would routinely read.

5.19 The high throughput assay that we developed significantly improved existing three dimensional gel assays because it provided a means to test many potential angiogenic molecules at once in a routine and standard manner. This assay was routinely being performed in my laboratory prior to March 1994 and in fact provided the means by which my research team were able to assay or screen large numbers of monoclonal antibodies for anti-angiogenic activity.

5.20 In addition to the tests that my staff and I were conducting as part of our research prior to 1994, we also provided some support to various researchers in South Australia and some researchers in other states of Australia, by testing for the presence of angiogenic activity in samples of material that they provided to us.

5.21 Although my research team and I never established any *in vivo* assays to measure aspects of angiogenesis, I was aware of a number of research groups around the world that were using such assays. At that time our research did not require the use of these assays since we were concerned with the action of factors directly on the endothelial cells rather than the overall process of angiogenesis that takes place *in vivo*. However should our work have evolved in such a direction then we would have established the relevant models. Many of these *in vivo* assays, although known to be labour intensive, once established could be performed by a competent research assistant.

5.22 In summary, prior to 1994, there were a number of assays available that could be, and were, routinely used to test the activity of putative and known angiogenic growth factors such as VEGF and PDGF.

6. The patent specification

6.1 I note that HGS succeeded in identifying a VEGF-2 gene and polypeptide sequence. In particular, the patent specification discloses that HGS identified a 1525 base pair nucleotide sequence, which they identified as translating to a 350 amino acid sequence (see: Figure 1 in the patent specification).

6.2 The patent specification teaches that the disclosed sequence contains the fourteen amino acid signature motif that is common to the PDGF/VEGF family of growth factors, as well as the eight cysteine residues which are conserved amongst VEGF, PDGFα, PDGFβ and PlGF family members (see paragraphs 5.1 to 5.13, above). By 1994, I knew from my own studies conducted on VEGF and PDGF, in addition to work published by others, that the signature motif and the cysteine residues in VEGF and PDGF were important for defining the biological activity of these molecules. Further, I was aware that the cysteine residues enable the PDGF/VEGF family of proteins to form dimers, which contributes to the biological activity of the molecule.

6.3 By March 1994 each of VEGF and PDGF were both known to undergo some proteolytic cleavage in their passage out of the cells in which they are produced. In particular, both molecules were known to be produced with a signal sequence which aides the passage of the molecules out of the cell. The processing of these molecules was also known to occur as

they are released from the cell and is important for producing a biologically active molecule.

6.4 I note that a portion of the full length VEGF-2 sequence, which is not disclosed in the patent specification, is part of the NH_2 (amino) terminal end of the full-length polypeptide sequence. This equates to 69 amino acids at the amino terminal end of the VEGF-2 polypeptide sequence. In all secreted proteins the signal or leader sequence is located at the amino terminal end of the molecule. VEGF-2 is no different in that it contains a signal or leader sequence at its amino end. This sequence is located among the 69 amino acids that were not disclosed in the patent specification.

6.5 The mere fact that the sequence disclosed in the patent application does not include the first 69 amino acids of the full-length VEGF-2 sequence would not dissuade me from attempting to express the sequence disclosed in the patent specification with a heterologous signal sequence. I would expect that the expressed protein would be secreted and biologically active. Although by 1994, the proteolytic processing of VEGF-2 had not been elucidated, the processing of related proteins VEGF and PDGF was well characterized. By 1994, it was known that members of the PDGF/VEGF family were proteolytically processed as they were secreted from the cell. Thus, I would predict that VEGF-2 would be expressed in a similar way. Additionally, the, biologically active forms of PDGF/VEGF proteins were known to retain their characteristic eight cysteine residues and 14 amino acid signature motifs. Thus, as the sequence disclosed in the patent specification contains both of these domains important for biological activity, I would expect that it would be correctly processed by the cell and secreted as a biologically active VEGF-2 protein. Dr Alitalo's own work has clearly confirmed that the sequence set forth in the patent specification combined with a heterologous signal sequence confers sufficient information to encode a secreted biologically active protein. (See: U.S. Patent No. 6,130,071, issued October 10, 2000, to Alitalo et al., column 47 line 57 to column 48 line 2).

Significance of the contribution provided by the patent specification

6.6 In my opinion, the identification of the DNA sequence encoding VEGF-2 and the inherent information it provides, combined with the teachings of

the patent specification, makes possible the manifest therapeutic benefits, which VEGF-2 has to offer, and which will be obtained in the future. The invention described in the patent specification embraces products and processes which have the potential to provide, and will continue to provide, therapeutic benefits to the public. The patent specification also provides the information necessary for production of a recombinant product with one or more of the biological properties of VEGF-2. It discloses methods for constructing various expression vectors suitable for use in a range of prokaryotic and eukaryotic host cells (*see e.g.*, pages 10 to 15 of the patent specification). It discloses host cells for expression, including eukaryotic mammalian cells, eukaryotic yeast cells, insect cell, and prokaryotic cells (*see e.g.*, pages 10 to 15 of the patent specification). By March 1994 all of this information was routinely available from standard text.

6.7 Further, I note that the patent specification teaches *in vitro* assays which I could have used as an assay system for testing for the biological function or activity of any protein I produced from the description in the patent specification. For example, on page 18 lines 6 to 8 the specification suggests assaying VEGF-2 by its ability to proliferate endothelial cells. This type of assay was so routine and well known in the field by 1994 that it was not necessary to provide any further details. Thus, the patent specification coupled with the state in the art as of March 1994 would enable one of skill in the art to assay to biological function or activity of VEGF-2. The patent specification did not need to describe specific experimental protocols to enable me to test for angiogenic activity in a recombinant protein.

6.8 In summary having regard to the state of knowledge associated with PDGF/VEGF proteins in March 1994, I believe the patent specification provides, amongst other information, the following:

6.8.1 The amino acid sequences of a range of different length VEGF-2 polypeptides;

6.8.2 The precise DNA sequence encoding the amino acid sequences identified in sub-paragraph 6.9.1;

6.8.3 Information identifying where VEGF-2 could be isolated from (eg a human cell library derived from human embryo (week 8 to 9) osteoclastomas, adult heart or breast cancer cell lines);

6.8.4 Methods for producing recombinant VEGF-2 in eukaryotic and prokaryotic host cells;

6.8.5 Ways for producing abundant amounts of a polypeptide isolated from recombinant cells having the *in vivo* activity of VEGF-2;

6.8.6 VEGF-2 polypeptide analogues;

6.8.7 VEGF-2 polypeptide antagonists, VEGF-2 polypeptide agonists;

6.8.8 VEGF-2 polypeptide antibodies;

6.8.9 Ways to test for the *in vitro* or *in vivo* biological function or activity of VEGF-2;

6.8.10 Pharmaceutical carriers and delivery systems for the VEGF-2 polypeptide as well as information concerning gene therapy to provide therapeutic and prophylactic effects against a wide range of different disease states; and

6.8.11 Uses of the VEGF-2 polypeptide and gene sequence for the treatment of numerous medical conditions.

6.9 At the conclusion of the patent application there are 61 claims, which define various aspects of the information that is described in the patent application.

Signal sequence

6.10 In March 1994 I was aware that angiogenic growth factors were secreted (such as the PDGF/VEGF family of growth factors including PDGFα, PDGFβ, VEGF and PlGF). Further, I knew that before an angiogenic protein could exert an angiogenic influence on a cell it needed to be released from the cell cytoplasm in which it was produced. Release of members of the PDGF/VEGF family was brought about by secretion of the respective member proteins.

6.11 Given that VEGF-2 was structurally similar in many ways to VEGF, PDGFα, PDGFβ and PlGF (eg it has eight cysteine residues and a 14 amino acid signature motif which is characteristic of these molecules (see: the patent specification page 5)), I would have expected that VEGF-2 was also a secreted protein.

Ludwig's Evidence in Support

7.1 I have been asked to read and comment on some of the statutory declarations by Ludwig's witnesses.

7.2 I have read:

7.2.1 The Statutory Declaration of Peter Adrian Walton Rogers executed on 16 February 2000 (Associate Professor Rogers' Statutory Declaration);

7.2.2 The Statutory Declaration of Kari Alitalo executed on 15 February 2000 ("Dr. Alitalo's Statutory Declaration"); and

7.2.3 The Statutory Declaration of Francis John Ballard executed on 16 February 2000 ("Dr. Ballard's Statutory Declaration").

7.3 In the following paragraphs I comment on some general issues raised in the above Statutory Declarations. I then turn to some specific comments made by each declarant. My decision not to address each paragraph in each Statutory Declaration should not be taken as an admission of acceptance of the paragraphs to which I do not refer.

7.4 Collectively, the declarants appear to suggest that the claims in the patent application cover molecules like VEGF, PDGFα, PDGFβ and PIGF. They appear to me to suggest that expression of the VEGF-2 sequence would have been problematic because of a variety of theoretical problems, none of which however appear to me to bear any relation to the case at hand.

7.5 There are essentially three issues raised by the Applicant's experts affidavits:

7.5.1 That the claims in the patent application cover molecules like VEGF, PDGFα, PDGFβ and PIGF because of the use of the words "fragments, analogues and/or derivatives";

7.5.2 That the patent specification fails to disclose a biological assay for testing for VEGF-2 or show biological activity of the identified VEGF-2 polypeptide sequence; and

7.5.3 That the patent specification does not identify a single antibody and conserved sequences between VEGF-2 and other PDGF/VEGF family members may bind cross reactive antibodies.

7.6 I deal with each of these issues in turn.

The claims in the patent application do not cover molecules like VEGF, PDGFα, PDGFβ and PlGF.

7.7 Reference is made on page 9, line 14 to page 10, line 5 of the specification to the meaning of the terms "fragment", "derivative" and "analog". When I read these passages I understood HGS to be saying that a fragment, derivative or analogue is a polypeptide that retains essentially the same biological function or activity as VEGF-2. While I acknowledge this basic requirement I am conscious of the fact that before something can be an analogue, fragment or a derivative of a protein it needs to share sufficient identity with that protein to make it resemble that protein or at least part thereof. In my opinion both of these requirements must be satisfied before a sequence of amino acids can truly be called an analogue, fragment or a derivative of VEGF-2.

(a) The need for biological function or activity

7.8 Provided a fragment, analogue or derivative has a biological function or activity then it should have some biological function or activity that is essentially the same as that observed for VEGF-2. When I read biological function or activity I immediately understand it to include at least *in vivo* and/or *in vitro* activity.

7.9 Testing for *in vivo* activity or *in vitro* activity of an angiogenic molecule like VEGF-2 would have been a routine exercise prior to March 1994. It was something that my laboratory could have easily conducted by 1994, since we were testing other angiogenic molecules like VEGF for *in vitro* activity.

7.10 There were a number of different assays available by 1994 capable of testing for VEGF-2 biological function or activity. For assays described in the specification I refer to and repeat paragraphs 5.14 to 5.22, above. For other assays that could have been performed to test for VEGF-2 biological function or activity I refer to and repeat paragraph 4.8 above.

7.11 As an additional point of distinction between VEGF-2 and PDGFα, or PDGFβ is that PDGFα and PDGFβ do not promote the growth of endothelial cells. Thus had I wanted to distinguish a VEGF-2 fragment, analogue or derivative from any one of these proteins, I could have easily

performed one or more assays, such as those identified in paragraph 5.15, above. Such assays could be performed to show that the analogue, fragment or derivative contained an endothelial cell proliferative activity, while the comparator molecule (such as PDGFα and PDGFβ) did not contain such an activity. Such a result would clearly have distinguished a VEGF-2 analogue, fragment or derivative from PDGFα, or PDGFβ.

(b) Must look like VEGF-2 at the primary amino acid level.

7.12 For something to be a fragment, derivative or analogue of a molecule it must not only share a biological function or activity with VEGF-2 but it must also have homology at the primary amino acid level. Thus, a peptide or polypeptide that more closely resembles VEGF, PDGFα, PDGFβ or PIGF, than VEGF-2 at the primary amino acid level would not be a VEGF-2 fragment, derivative or analogue. Such an interpretation is inherent in the meaning of these terms and is consistent with the general manner in which others and I use these terms in everyday scientific language.

7.13 When regard is had to the information provided in the patent specification one observes that the homologies at the amino acid level between VEGF, PDGFα, PDGFβ and VEGF-2 are very low. The identity between VEGF-2 and each of VEGF, PDGFα, PDGFβ is 30%, 23% and 22% respectively (see page 5 of the patent specification). Further, there are very few contiguous sequences of amino acids that are identical between VEGF-2 and each of VEGF, PDGFα or PDGFβ (see Figure 2 of the patent specification) In fact, the largest single contiguous sequence of amino acids is only seven amino acids in length and is found in the signature motif, which is identified by a box in Figure 2. After that stretch of residues there is one other stretch of six contiguous amino acids but no other significant contiguous amino acid sequences that share identity between VEGF-2 and VEGF, PDGFα or PDGFβ. Thus, at the primary amino acid level there are significant differences between VEGF-2 and VEGF, PDGFα or PDGFβ.

7.14 I would not have any problem in distinguishing between a fragment, derivative or analogue of VEGF-2 and a fragment, derivative or analogue VEGF, PIGF, PDGFα or PDGFβ especially taking into account both biological activity and sequence homology. If the fragment, analogue or derivative has a higher homology to VEGF-2 than VEGF, PIGF, PDGFα or PDGFβ and shares a biological activity also present with the sequence disclosed in the patent application then I would consider it a VEGF-2 fragment, analogue or derivative.

The patent specification provides sufficient information to test for VEGF-2 biological function or activity.

7.15 The patent specification clearly identifies that VEGF-2 has angiogenic activities, and describes *in vitro* angiogenic assays of VEGF-2, which could be routinely used. Furthermore, by 1994 assays for angiogenic biological activity were widely reported in the literature. In this respect I refer to Litwin, Gamble, Vadas, 1995, "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129, which identifies many publications that were available prior to 1994 that describe assays for testing many different angiogenic activities.

7.16 Had I been provided with a copy of the patent specification in March 1994 I would not have required anyone to tell me how to carry out an assay to test for an angiogenic activity. I refer to and repeat paragraphs 5.14 and 5.22 above which address this is in more detail.

7.17 I note that Ludwig's experts and in particular Associate Professor Rogers observe that HGS has not tested the biological activity of the protein that they describe in their patent application. However, confirmation that the VEGF-2 sequence described in the specification is expressed *in vivo*, and therefore likely to indicate a biological activity, is provided in the Examples of the specification where it is shown by Northern blotting that a number of malignant breast tumour cell lines over express VEGF-2. The over expression of VEGF-2 in breast cancer cell lines while absent in a normal breast sample suggests a role of VEGF-2 in tumor development perhaps by promoting the growth of new blood vessels, as was observed for VEGF. I refer to and repeat paragraphs 5.10. Other assays to further characterise biological activity, such as *in vitro* tests using purified VEGF-

2 protein, would have been straight forward for any laboratory that was set up to perform such assays. I refer to and repeat paragraphs 5.14 to 5.22 above which address this is in more detail.

7.18 Further, whether or not the patent specification gives examples of biological activity would seem to me to be largely irrelevant. If I were told that a molecule had angiogenic activity but there were no experiments present in that document to support that statement I would, in March 1994, have proceeded to test the molecule myself. Moreover, even if such data were provided I would probably still have conducted such experiments, because they were simple to carry out and routinely performed in my laboratory.

The patent specification provides sufficient information for VEGF-2 antibodies.

7.19 Associate Professor Rogers' asserts that the patent specification fails to indicate that the inventors actually made any anti-VEGF2 antibodies.

7.20 I believe the patent specification provides sufficient information for a person of ordinary skill to produce VEGF-2 antibodies (see pages 22 to 24). Methods for generating antibodies against a protein were very well known by March 1994. The patent specification identifies on page 23 a number of publications that describe such methods that were well known to me and had been used in my research prior to March 1994. In my opinion, a person of ordinary skill in the field of immunology should be able to produce antibodies against VEGF-2 with ease.

Cross reactivity of VEGF-2 antibodies with other proteins

7.21 Associate Professor Rogers appears to suggest (see, for example paragraph 2.7.16) that antibodies that bind to regions of VEGF-2 that are conserved with other PDGF/VEGF family members might be cross-reactive. Associate Professor Rogers refers to a number of prior art disclosures of anti-VEGF or anti-PDGF antibodies. Simply because two sequences share some homology does not necessarily imply that any antibody produced against one molecule would necessarily be cross-reactive against the other. In my opinion no conclusion can be made as to whether sequences sharing some homology will generate antibodies that are cross reactive.

(I) Associate Professor Rogers' Statutory Declaration

7.22 I refer to Associate Professor Rogers' Statutory Declaration, and make the following comments.

Paragraph 1.5.3

7.23 In paragraph 1.5.3, Associate Professor Rogers' states:

> "...extensive proteolytic processing occurs at both the amino- and carboxyl-terminal ends of the polypeptide, resulting in a much smaller circulating polypeptide that has enhanced and/or new biological activities relative to the larger pre-processed forms from which it was derived... ...However, in the opposed application filed by 1994, there is no evidence presented of complex proteolytic processing."

7.24 However, I was aware that all members of the PDGF/VEGF family (known in March 1994) underwent some proteolytic processing when produced from a cell. Thus, had I been asked to examine VEGF-2 in March 1994 I would have expected that VEGF-2 may also naturally undergo some proteolytic processing when released from a cell. This process occurs naturally during secretion, controlled by cellular enzymes. Consequently, if a researcher were able to produce VEGF-2 and secrete it from a cell I believe a reasonable expectation would be that processing would take place. Therefore, in my opinion, the patent specification fully enables one to express and secrete a biologically active protein that has been correctly processed.

Paragraph 2.2

7.25 In paragraph 2.2, Associate Professor Rogers' mentions that several claims in the patent specification are directed to a "fragment, analogue or derivative" of a VEGF-2 polypeptide (*e.g.*, claim 28) or to a polynucleotide encoding a "fragment, analogue or derivative" of a VEGF2 polypeptide (*e.g.*, claims 1 to 4 and 21). He then proceeds to state:

> "...all of the differences between the VEGF2 sequence and prior art sequences such as the human VEGF sequence can properly be characterized as modifications that involved substituting, adding, or deleting residues, the same types of modifications taught in the opposed application for making fragments, analogues, or derivatives."

7.26 I disagree with Associate Professor Rogers' statement. If a molecule has higher homology to VEGF than VEGF-2 then it is not in my opinion an analogue of VEGF-2.

7.27 Thus, I would not regard VEGF or PDGF or PIGF to be an analogue or derivative of VEGF-2, nor do I believe that a researcher of reasonable skill would have any problem in distinguishing between VEGF and VEGF-2 or a fragment, analogue or derivative thereof.

Paragraph 2.3.3

7.28 In paragraph 2.3.3, Associate Professor Rogers' states:

> "For example, there is no description of tests that were performed to demonstrate that VEGF2 has a biological activity that is useful in such procedures, and there is no description of any assay to test for VEGF2 biological activity."

7.29 As discussed in paragraph 7.17 above, I consider the Northern blots described in the Examples of the specification to be indicative of a biological activity for VEGF-2. Further, I do not agree that the patent application does not identify a means to test for VEGF2 biological function or activity. In this respect I refer to page 18 of the patent specification where I note the patent specification clearly states that VEGF-2 can be used for *in vitro* culturing of vascular endothelial cells. I recognise this as the basic cell proliferation assay and could have used this assay to test for VEGF-2 activity. It was not, however, the only assay available at that time for testing activity of molecules, like VEGF-2. There were many other assays some of which are described in paragraph 5.15 above.

Paragraph 2.7.18

7.30 In paragraph 2.7.18, Associate Professor Rogers' comments on the scope of claim 50. I particular he states:

> "Assuming *arguendo* that VEGF2 as taught in the specification possesses any biological activity that is mediated through cell surface receptors, the claims directed to antagonists of VEGF2 are not novel over prior art disclosures of forms of the receptors to which VEGF2 could bind, but could not signal. See **Document D27** (disclosing a dominant negative Flk-1 protein)."

7.31 When I read claim 50 I note that it requires the defined antagonist to be specific for the polypeptide of claims 28 to 48. Associate Professor Rogers then cites Document D27 that describes a dominant negative Flk-1 protein. There is no evidence in that publication, which establishes that the identified truncated receptor is capable of binding specifically to VEGF-2. I am aware from research published since 1994 that the Flk-1 receptor, when produced in a non-truncated form, binds VEGF as well as VEGF-2. Thus the truncated Flk-1 protein described in Document D27 is not specific for VEGF-2, as the claim appears to me to require.

Paragraph 2.7.19

7.32 In paragraph 2.7.19, Associate Professor Rogers' states:

> "Because of this alleged equivalence of activities taught in the opposed application, patients in need of VEGF2 (or in need of inhibiting VEGF2) would be treatable with VEGF (or with VEGF antagonists). Consequently, claims 51 and 52 embrace any prior art method of treatment of patients with VEGF polypeptides that are encompassed by claim 28 (or prior art method of treatment with VEGF antagonists)."

7.33 As I read claims 50 and 51 I note that they are directed to a method of treatment of a patient having need of VEGF2 or need to inhibit VEGF2 by administering an effective amount of a polypeptide according to claim 28 or an antagonist against a polypeptide of claim 28. I do not understand how either of these claims could possibly embrace the subject matter mentioned by Associate Professor Rogers. In particular, in my opinion the claims in the patent specification appear to be directed to use of VEGF-2 not VEGF and to the treatment of a VEGF-2 disorder. While VEGF-2 and VEGF may be used to treat some of the same disorders I note that HGS appear to me to have only claimed the use of VEGF-2.

Paragraph 2.7.20

7.34 In paragraph 2.7.20, Associate Professor Rogers' states:

> "I observe that the opposed application fails to identify with particularity any fragment, analogue, or derivative of the Figure 1 polypeptide which has an inhibitory activity."

7.35 I and I believe other researchers of ordinary skill in the molecular biological field could easily generat[illegible] [illegible]agments, analogues or derivates of VEGF2 and could have tested them at March 1994 using any one of a

large number of different biological assays to detect inhibitory activity. In fact, in collaboration with other groups in the Hanson Centre for Cancer Research, we had been doing similar experiments at that time although not with VEGF2. Therefore, by providing VEGF-2 coding sequences, the patent specification provided all of the information necessary for me and I believe any one else of ordinary skill to identify a fragment, analogue, or derivative of the Figure 1 polypeptide that has an inhibitory activity.

Paragraph 4.6.5

7.36 In paragraph 4.6.5, Associate Professor Rogers' states:

> "To summarize, the opposed application contains no demonstration of VEGF2 activity and there is no reasonable basis upon which one skilled in the art could have predicted VEGF2 activity."

7.37 I refer to and repeat paragraphs 5.14 to 5.22, 7.8 to 7.11 and 7.15 to 7.18, which address this issue.

(ii) <u>Dr. Alitalo's Statutory Declaration</u>

7.38 I refer to Dr. Alitalo's Statutory Declaration, and make the following comments.

7.39 In 1996, Dr Alitalo first described a VEGF-C polypeptide sequence that was 350 amino acids long, not unlike the sequence provided in the patent specification. A copy of Dr Alitalo's publication is identified as **Document D70,** in his statutory declaration. In that publication the authors state on page 291 (first column):

> "The two longest clones of 2.0 and 1.8 kb contained an open reading frame (ORF) of 350 residues shown in Figure 3B, having two possible methionine codons (marked in bold) for translation initiation and a putative secretory signal sequence peptide (underlined) followed by the N-terminal sequence of the purified Flt4 ligand (marked in Bold)".

7.40 Further, on page 293 (first and second columns) the author's state:

> "Interestingly, the VEGF-C ORF is 350 amino acid residues long and our N-terminal sequence analysis confirmed that its putative signal sequence is removed before secretion."

7.41 Shortly after publishing these results Dr. Alitalo published a correction to that paper (see Joukov *et al.*, 1996, EMBO Journal 15: 1751). Thus, at the time of writing his statutory declaration, I would have expected Dr. Alitalo to be aware of the correct signal sequence from VEGF-C. Since he followed a similar path to that which HGS seems to have followed in the patent specification when attempting to characterise what he thought was the full length VEGF-2 sequence, he knew that the signal sequence identified in the specification was not such a sequence.

Paragraph 6.5

7.42 Dr. Alitalo's experimental protocol indicates that he employed two constructs, one encoding amino acids 1 to 419 of VEGF-C (VEGFC), and a second encoding amino acids 70 to 419, followed by an HA tag (VEGF2 (HGS)). In paragraph 6.5 of his declaration, Dr. Alitalo indicates that he used a monoclonal anti-HA antibody to detect VEGF2(HGS) and another antibody serum, 882 antiserum, which Dr Alitalo indicates is a polyclonal antiserum generated against residues 35-51 of the 350 amino acid VEGF2 polypeptide to detect VEGF-C.

7.43 I do not believe that any conclusions can be drawn from the experiments conducted by Dr. Alitalo because he used different antibodies to examine the VEGF2(HGS) protein and VEGF-C. The scientifically most meaningful method used to compare the secretion of VEGF2(HGS) protein and VEGF-C would be to use the identical antibody to immunoprecipitate the two proteins. Since the antibody used to immunoprecipitate VEGFC should also recognize VEGF2 then this should have been used. Alternatively, if the method of choice was to immunoprecipitate via a HA tag, then this tag should have been incorporated into both VEGF-C and VEGF2.

7.44 The second problem with the design of the experiments is a comparison made between HA tagged and non-HA tagged proteins. It has not been demonstrated that the HA tagging has not altered the capacity of the protein to be synthesised and secreted or detected. Indeed, in his own publication (Joukov et al., 1997, EMBO J. 16:3898-3911) Dr Alitalo and his colleagues have stated that they were unable to immunoprecipitate secreted VEGF-C with an antibody directed to a HIS-tag incorporated into the very C terminal end of the protein although they were able to do this when a HIS-tag was placed immediately behind the signal sequence.

Given the information now known and known at the time of designing this experiment it would appear to me that VEGF C/2 is highly processed with the very C terminal end of the protein removed.

7.45 In HGS' Australian Patent No. 714484, a monoclonal anti-HA antibody was used to successfully immunoprecipitate VEGF-2 which had been modified to contain an HA epitope at its carboxy terminus. It is unclear to me why Dr. Alitalo apparently was not able to isolate VEGF-2 using a His tag at the C-terminus. One explanation may be the type of mammalian cell line used in the experiments. The modified VEGF-2 was expressed from COS cells in HGS' experiments. However, Dr. Alitalo used 293T cells in the experiments reported in his declaration. The significance of the different cell types used by HGS and Dr. Alitalo can be found in Dr. Alitalo's own publication (Joukov et al., 1997, EMBO J. 16:3898-3911). This publication describes the proteolytic processing of VEGF-2 when expressed by a number of different cell lines, including COS cells, PC-3 cells, HT1080 cells and 293-EBNA cells. The result of this comparison was that "[t]he proteolytic processing of the VEGF-C precursor in COS cells was less efficient when compared with other cell types" (see: Joukov at page 3901, second column). Thus, as the VEGF-2 precursor is processed less efficiently in COS cells, one would also expect that the cleavage of the HA tag from the carboxy terminus would also be less efficient in COS cells, as compared to 293T cells. So, that the lack of efficient cleavage of the HA-tag from the protein may account for HGS' successful detection of the protein utilizing the HA-tag from COS cells, while Dr. Alitalo was unable to do so.

7.46 Assuming, however, that the ultimate conclusion reached in these experiments is correct, i.e. there is no secretion signal sequence in the 350 amino acid sequence disclosed in the patent specification, such a result would not have stopped me from attempting to produce a biologically active VEGF-2 polypeptide sequence using the information in the patent specification and knowledge available to those skilled in my field by March 1994.

7.47 Using the knowledge that PDGF/VEGF family members are secreted molecules and using the sequence information in the patent specification I would have reasoned that the failure of the VEGF-2 protein to be secreted may be due to an inefficient or atypical signal sequence or a

missing or partial signal sequence. Another possibility is that the VEGF-2 protein is indeed not secreted. However given that all the other PDGF/VEGF family members known prior to March 1994 were secreted molecules, I would have regarded this as an unlikely explanation.

7.48 Given the weight of evidence that VEGF-2 is indeed a secreted protein, I believe a more reasonable explanation for the lack of secretion of VEGF-2 is an inefficient signal sequence or a missing or partial signal sequence. Thus, this explanation would have prompted me to attach a strong signal sequence upstream from the nucleotide sequence encoding VEGF-2, as taught by the patent specification (at page 14 lines 6-23). Using this approach I would have fully expected to achieve expression and secretion of the VEGF-2 protein. Furthermore, as discussed above in section 6, the VEGF-2 sequence provided in the patent specification provides all the information required to encode a biologically active protein. Thus I would have proceeded using the approach provided by the patent specification and having done so, I would fully expect the VEGF-2 protein to be secreted <u>and</u> biologically active.

7.49 In addition, since as mentioned above, the lack of secretion may be due to a missing or partial signal sequence, I would have been motivated to confirm whether there was indeed more sequence information at the amino end of the molecule that was missing from the initial cDNA clone.

Paragraphs 7.2 & 8.3

7.50 In paragraph 7.2 Dr Alitalo states:

> "...it is readily apparent from the autoradiogram that the expression level of VEGF-C is much higher than that of VEGF2(HGS)".

7.51 Further, at paragraph 8.3 Dr Alitalo states:

> "The fact that VEGF-C expression observable in cell lysates of VEGF-C-transfected cells is much higher than VEGF2(HGS) expression observable in VEGF2(HGS)-transfected cells suggests that VEGF2(HGS) is inefficiently translated and/or that the intracellular turnover rate of VEGF2(HGS) is much faster than that of VEGF-C. In other words, the cells may be recognizing VEGF2(HGS) as an aberrant protein and rapidly degrading it."

7.52 In my opinion Dr. Alitalo's conclusions represent pure speculation and cannot reasonably be drawn from the results presented in his statutory

declaration for two reasons. First, Dr. Alitalo used two different antibodies to compare the VEGF2(HGS) protein and VEGF-C. Moreover, he has not established that the detection capacity of these two antibodies is the same. Second, the position of incorporation of the HA tag into the VEGF2 construct would appear to be in a position that Dr Alitalo himself has shown to result in inefficient immunoprecipitation of active secreted VEGF C.

(ii) ***Dr. Ballard's Statutory Declaration***

7.53 I refer to Dr. Ballards' Statutory Declaration, and make the following comments.

7.54 Dr Ballard's Statutory Declaration merely confirms the statements made in the Statutory Declarations by Associate Professor Rogers and Dr. Alitalo. I repeat my comments above in response to this Statutory Declaration

AND I make this solemn declaration by virtue of the Statutory Declarations Act, 1959 and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DATED this Twelfth day of December 2000.

DECLARED at: Geneva)

BEFORE me: Pierre CHRISTEN)

JENNIFER RUTH GAMBLE

Commissioner of Declarations/patent attorney/Justice of the Peace/Solicitor

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12th December 2000.-

TIMBRE CANTONAL Fr. 2.50

PIERRE CHRISTEN NOTAIRE à GENÈVE

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure JRG-1** referred to in my Statutory Declaration made this Twelfth day of December 2000.

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12th December 2000/mb.-

JRGamble

Jennifer Ruth Gamble

TIMBRE CANTONAL

PIERRE CHRISTEN NOTAIRE à GENÈVE

WITNESS: ______________________

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

CURRICULUM VITAE

NAME:	**GAMBLE**, Jennifer Ruth
BORN:	4 September, 1952
QUALIFICATIONS:	1978 B. App. Sc. (RMIT, Victoria) 1986 M.Sc. (University of Melbourne) 1994 Ph.D. (University of Adelaide) 1998 Associate Professor (University of Adelaide)
CONTACT:	Telephone: (61 8) 8222-7482 Facsimile: (61 8) 8232 4092 E-mail: Jennifer.Gamble@imvs.sa.gov.au

PRESENT APPOINTMENT:

Research Scientist, Institute of Medical & Veterinary Science, Adelaide
Founding Member, Hanson Centre for Cancer Research, IMVS, Adelaide
Co-Head, Vascular Biology Unit, Hanson Centre for Cancer Research
Associate Professor, Department of Medicine, University of Adelaide

PREVIOUS APPOINTMENTS:

1971-79	Research Assistant, Experimental Pathology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne
1979-80	Visiting Research Assistant at Institute for Immunology, Marseille, France
1979-85	Research Assistant, Thymus Biology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne
1985	Visiting Scientist, University of Washington, Seattle, USA
1985-92	Research Scientist, Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide
1991	Visiting Scientist, Scripps Research Institute, San Diego, USA.
2000-2001	Visiting Professor, University of Geneva, Switzerland

MEMBERSHIP OF PROFESSIONAL SOCIETIES:

Inaugural member of Australian Vascular Biology Society

COMMITTEES:

National Health & Medical Research Council of Australia, Regional Grants Committee	1997, 1998, 1999
National Heart Foundation, Australia, Regional Grants Committee	1997, 1998, 2000
National Heart Foundation, Australia, Fellowship Committee	1999
University of Adelaide, Division of Health Sciences Research Committee	1996-2000
Women's & Children's Hospital, Adelaide, Research Grants Committee	1996-1998

REFEREE FOR GRANTING BODIES:

National Health and Medical Research Council of Australia
National Heart Foundation, Australia
Anti-Cancer Foundation of the Universities of South Australia
Anti-Cancer Foundation of Victoria
Anti-Cancer Foundation of New South Wales
Western Australia Cancer Foundation
Arthritis Foundation of New South Wales
Arthritis Foundation of Victoria
Various University and Hospital Research Committees

REFEREE FOR JOURNALS:

Journal of Cell Biology
Journal of Clinical Investigation
Journal of Immunology
Immunology and Cell Biology
Growth Factors
Journal of Leukocyte Biology
Laboratory Investigation
Journal of Vascular Research
Atherosclerosis, Thrombosis & Vascular Biology
Thrombosis Research

THESIS EXAMINER:

University of New South Wales
University of Queensland
University of Adelaide
University of Sydney

CONFERENCE ORGANISATION:

Australian Vascular Biology Society Scientific Meeting	1994 and 1999
Hanson Centre for Cancer Research Symposium	1994, 1996, 2000

GRANTS HELD

(Previous):

Australian Brain Foundation M Vadas, G Burns, A Lopez, J Gamble
"Endothelium and white blood cell interaction of the pathogenesis of cerebrovascular disease"
1986 $5000

National Health & Medical Research Council (No. 860756) M Vadas, J Gamble
"Regulation of granulocyte endothelial interactions in man"
1986-1988 $54,750, $42,732, $42,732

Anti-Cancer Foundation of the Universities of South Australia G Burns, D Gillis, J Gamble
"Molecules mediating adhesion of tumour cells: their role in distribution and metastatic spread"
1987 $24,000

Anti-Cancer Foundation of the Universities of South Australia A Lopez, J Gamble, LB To
"Identification of a novel growth factor for human hemopoietic cells: biological and molecular characterisation" 1987 $25,000

National Heart Foundation M Vadas, J Gamble
"Role of TNF-α and IL-1 in thrombotic phenomena" 1987-1988 $37,594 & $33,069

Anti-Cancer Foundation of the Universities of South Aust A Lopez, JR Gamble, LB To
"Regulation of leukaemia cell proliferation and differentiation by recombinant human IL-3"
1988-1989 $24,000 pa

National Health & Medical Research Council (No. 980844) MA Vadas, JR Gamble
"Regulation of endothelial adhesiveness for blood cells in man"
1989-1991 $51,000 $52,000 $53,000

Anti-Cancer Foundation of the Universities of South Australia JR Gamble, MA Vadas
"*In vitro* models of angiogenesis: investigations of events involved in capillary invasion of neoplasms" 1990-1991 $32,764 $37,400

National Heart Foundation MA Vadas, JR Gamble, PJ Nestel
"Regulation of monocyte-endothelial cell interactions" 1990-1992 $33,000 pa

National Health & Medical Research Council (No. 920866) MA Vadas, JR Gamble
"Mechanism of action of proteins that inhibit neutrophil-endothelial interactions"
1992-1993 $87,320 $91,994 $91,994

National Health & Medical Research Council (No. 920867) JR Gamble, P Kaur
"Cell surface antigens involved in angiogenesis" 1992-1993 $65,217 & $66,227

Anti-Cancer Foundation of the Universities of South Australia JR Gamble, P Kaur
"Purification of novel factors involved in the process of new blood vessel formation (angiogenesis)" 1994 $41,900

Royal Adelaide Hospital "β1 antigens" JR Gamble 1994 $10,000

The Kathleen Cuningham Foundation for Breast Cancer Research JR Gamble
"Dominant mutant retinoblastoma gene product as a potential regulator of angiogenesis"
1996-1997 $30,000 pa

National Heart Foundation J Gamble and P Xia
"New Mechanism of Atheroprotection: High Density Lipoproteins, Endothelial Adhesion Molecule Expression and the Sphingomyelin Pathway" 1998-1999 $47,000 pa

Anti-Cancer Foundation of South Australia J Gamble and B Wattenberg
"Lumen Formation in Angiogenesis: Characterisation of the membrane targetting molecules that regulate intracellular vesicle fusion as a prerequisite for lumen formation" 1999 $47,000

(Current):

National Health & Medical Research Council Program Grant
M Vadas, A Lopez, J Gamble, P Cockerill, G Goodall
"Leukocyte and Endothelial Cell Biology" 1997-2001 $928,846 pa

National Health & Medical Research Council Large Equipment Grant
J Gamble, L Ashman, D Findlay and M Vadas $22,000

National Heart Foundation P Xia and J Gamble
"A Novel Signalling Pathway in Atherosclerosis" 2000-2001 $45,000 pa

Faculty of Health Sciences, University of Adelaide J Gamble and M Vadas
Equipment Grant, 2000 $48,000

Rebecca Cooper Medical Research Foundation J Gamble
"Role of Sphingosine Kinase in Arthritis" 2000 $7,500

PATENTS

A Method of Modulating Cellular Activity. (PCT/AU98/00730) Provisional file, September 1997. Vadas MA, Gamble JR, Xia P, Barter P, Rye K-A, Wattenberg B and Pitson S.

A Novel Cation Chloride Cotransporter Molecule and genetic sequences encoding same. (PP7008/98) Provisional file, November 1998. Medvet Science Pty Ltd. Hiki K, Gamble JR, Vadas MA, D'Andrea R and Sutherland G.

Novel therapeutic molecules and uses thereof - I. Provisional Patent (PQ0339/99) 13 May 1999. Pitson SM, Wattenberg BW, Xia P, D'Andrea RJ, Gamble JR, Vadas MA. App No PQ8408/00.

Novel therapeutic molecules and uses thereof - II. Provisional Patent (Q1504/99) 8 July 1999. Pitson SM, Wattenberg BW, Xia P, D'Andrea RJ, Gamble JR, Vadas MA.
(I and II Combined: PCT/AU00/00457).

INVITED SPEAKER:

Australian Society for Medical Research, Adelaide 1991
Baker Symposium, Melbourne 1992
Annual Inflammation Symposium, Sydney 1993, 1994
Australian Vascular Biology Society Annual Meeting 1993, 1995, 1997

Hanson Centre for Cancer Research Symposium	1994,
1996	
Asia Pacific League of Associations of Rheumatology Meeting, Melbourne	1996
International Vascular Biology Society Meeting, Seattle, USA	1996
Lorne Cancer Meeting	1997
International Vascular Biology Society Meeting, Cairns, Australia	1998
Keystone USA Symposium "The Endothelium"	1998
Australasian Haematology Society, Adelaide	1998

SYMPOSIUM CHAIRPERSON

Australian Vascular Biology Society Meeting	1994,
1996	
International Vascular Biology Society, Seattle, USA	1996
International Vascular Biology Society, Cairns, Australia	1998

PUBLICATIONS:

1. Basten, A., Miller, J.F.A.P., Warner, N.L., Abraham, R., Chia, E. and GAMBLE, J. (1974) A subpopulation of T cells bearing Fc receptors. Journal of Immunology 115:1159-1165.

2. Miller, J.F.A.P., Vadas, M.A., Whitelaw, A. and GAMBLE, J. (1975) H-2 gene complex restricts transfer of delayed-type hypersensitivity in mice. Proceedings of the National Academy of Sciences USA 72:5095-5098.

3. Miller, J.F.A.P., Vadas, M.A., Whitelaw, A. and GAMBLE, J. (1975) A radioisotopic method to measure delayed type hypersensitivity in the mouse. II. Cell transfer studies. International Archives of Allergy and Applied Immunology 49:693-708.

4. Vadas, M.A., Miller, J.F.A.P., GAMBLE J. and Whitelaw, A. (1975) A radioisotopic method to measure delayed type hypersensitivity in the mouse. I. Studies in sensitized and normal mice. International Archives of Allergy and Applied Immunology 49:670-692.

5. Basten, A., Miller, J.F.A.P., Abraham, R., GAMBLE, J. and Chia, E. (1975) A receptor for antibody on B lymphocytes. III. Relationship of the receptor to immunoglobulin and Ia determinants. International Archives of Allergy and Applied Immunology 50:309-321.

6. Miller, J.F.A.P., Vadas, M.A., Whitelaw, A. and GAMBLE, J. (1976) Role of major histocompatibility complex gene products in delayed type hypersensitivity. Proceedings of the National Academy of Sciences USA 73:2486-2490.

7. Miller, J.F.A.P., Vadas, M.A., Whitelaw, A. and GAMBLE, J. (1976) H-2 linked Ir gene regulation of delayed-type hypersensitivity in mice. In "The Role of products of the Histocompatibility Gene Complex in Immune Responses". DH Katz and B Benacerraf eds., pp. 403-415. Academic Press, NY.

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COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure JRG-2** referred to in my Statutory Declaration made this Twelfth day of December 2000.

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12th December 2000/mb.-

Jennifer Ruth Gamble

TIMBRE CANTONAL

Fr. 250

PIERRE CHRISTEN NOTAIRE à GENÈVE

WITNESS: ____________________

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

Summary Research History

Jennifer Ruth Gamble

1.1. From 1971 to 1979 I was a Research Assistant with the Experimental Pathology Unit at the Walter Eliza Hall Institute for Medical Research. During that period in time I studied the role of the histocompatibility complex in immune responses concentrating on delayed-type hypersensitivity reactions.

1.2. Between 1979 and 1980 I was a Visiting Research Assistant at the Institute for Immunology in Marseille in France. I also held the position of Research Assistant with the Thymus Biology Unit at the Walter Eliza Hall Institute for Medical Research in Melbourne. It was during this period that I was investigating the biological and biochemical nature of the T Cell Receptor.

1.3. In 1985, I took a sabbatical in Seattle at the University of Washington where I started to learn the techniques of endothelial cell isolation and began to examine endothelial cell function. In particular, I studied the adhesion of inflammatory cells such as neutrophils to the endothelium. While carrying out these studies I obtained a sample of TNF-α, which had recently been cloned at Genentech and used it in the controls in the experiments that I was conducting. From those experiments I established that endothelial cells could be regulated by inflammatory cytokines like TNF-α. This was the first description that the phenotype of endothelial cells could be altered. The results of that research were published in 1985 in publication 22 in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2a**" is a true copy of publication 22. That article is widely quoted in the area of inflammation. During and after that period I investigated the effects of a number of cytokines on the function of the endothelium and inflammatory cells. These cytokines included GM-CSF, IL4, IL5 and IL3.

1.4. Towards to the end of 1985 I moved to the Institute of Medical and Veterinary Science (IMVS) in South Australia, where I remain today. Upon arriving in Adelaide I established the Vascular Biology Unit at the IMVS. My research at that time continued to look at the regulation of endothelial cell function with particular emphasis on adhesion molecules and inflammatory type responses in endothelial cells.

1.5. Between 1985 and about 1990 I studied the regulation of adhesiveness of endothelial cells. It was during this period that I discovered that the cytokine TGF-β inhibited TNF action. That research was published in the prestigious journal *Science* in 1988 and is identified as publication 33 in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2b**" is a true copy of publication 33. Thus, I had demonstrated that adhesion molecule expression on endothelial cells could be both stimulated (eg by TNF) and inhibited (eg by TGF).

1.6. I have continued to investigate the adhesive properties of the endothelium. However about 1991, my colleagues and I started to consider the role of endothelial cells in angiogenesis. Therefore we established assays to measure endothelial cells undergo angiogenesis. An *in vitro* assay system that measured capillary tube formation, which is classically referred to as the gel-type assay was established since this measured many different aspects of angiogenesis.

1.7. The major difference between the assay that I set up and the gel-type assays that were routinely carried out by other research groups at that time was that my assay was performed in microtitre wells. This was developed as a means to screen large numbers of molecules under different biological conditions for pro-angiogenic or inhibitory functions. The assay was reported in 1993 in publication 51 identified in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2c**" is a true copy of publication 51. The same publication was also one of the first reports showing that cell surface molecules called integrins are involved in angiogenesis.

1.8. By about 1991 my laboratory was using three different assays to measure aspects of angiogenesis *in vitro*. They were the cell proliferation assay, improved gel type assay and a wound assay. In the wound assay, a monolayer of endothelial cells is damaged by way of a scrape and measurement is made of the ability of the damaged monolayer to repair itself.

1.9. By 1991 VEGF was also starting to become the focus of a great deal of scientific research. While VEGF had been postulated to exist well prior 1991, it was around 1991 that Genentech first described the cloning of the molecule.

1.10. Shortly after Genentech cloned VEGF I received some samples of the molecule to study. Since my return from the University of Washington in 1985 I had maintained my collaboration with Genentech. They knew that I had set up a number of assays for measuring endothelial activity. Hence we extended our collaboration with them to investigate the biological activity of VEGF.

1.11. When I obtained the Genentech VEGF material I began to look at various aspects of VEGF activity together with another angiogenic cytokine molecule that was also being described at that time, namely Fibroblast Growth Factor (FGF). Initially both VEGF and FGF were examined using the proliferation assay that I had set up. Those studies provided a quick and easy way to confirm that the material that I had obtained from overseas was in fact active, since both VEGF and FGF were known to be mitogens. I then set out to study the activity of these cytokines in the gel assay that I had developed. My gel assay studies examined the activity that both FGF and VEGF displayed in the angiogenic process. I also looked at the effects of FGF and VEGF on molecules such as the integrins and other signalling molecules that were known to be important in the process of angiogenesis. These studies were carried out to determine the activity of the growth factors, how they influenced other molecules and how they modulated the extent and characteristics of angiogenesis.

1.12. In addition to the abovementioned studies my colleagues and I also started, in 1993, to examine the morphology of the endothelial cells as they were starting to form capillary tubes. In 1997, my colleagues and I described, for the first time, how lumina form during angiogenesis. That research was published in publication 73 identified in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2d**" is a true copy of publication 73. However, still to date little is known of the molecular mechanisms or events that are needed to reorganize endothelial cells to provide their three-dimensional structure.

1.13. While conducting our morphology studies I also initiated a small project to look for inhibitory molecules of the process of angiogenesis. That research, which I carried out between 1994 and 1996, sought to identify antibodies that bound to endothelial cells which blocked the process of angiogenesis. In that research, monoclo[illegible] antibodies were generated against the endothelial cell surface. The monoclonal antibodies were then screened

using the gel assay that I had developed, to test whether any were capable of inhibiting angiogenic activity. While we were able to produce hundreds of different monoclonal antibodies, we were not able to identify a specific antibody that was particularly attractive in its anti-angiogenic activity.

1.14. I also started to examine the regulation of cell junctions, which we postulated at that time, might be regulated by VEGF. For endothelial cells to make a new blood vessel they need to break their existing cell junctions (interactions) with their neighbours thus allowing them to migrate and proliferate. Inhibition of angiogenesis would suggest the reforming of tight junctions. Publication number 72 in my *curriculum vitae* demonstrates that junctional molecules are not only structural but also provide important signals to the endothelial cell that has consequences to the phenotype of the cell. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2e**" is a true copy of publication 72.

1.15. After my colleagues and I published our finding in publication number 72 in my *curriculum vitae* we went on to determine that VEGF165 alters these junctional molecules consistent with the hypothesis that the tightness of the junction would be decreased. We also showed that VEGFB and VEGF121 induced similar changes. Further, we also showed that no major differences were seen between the three forms of VEGF in the assays performed which included capillary tube formation in collagen gels, permeability induction and proliferation.

1.16. More recently we have determined that another "angiogenic factor" Angiopoietin1 is able to inhibit the VEGF mediated induction of permeability and changes the junctional molecules involved in permeability. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2f** "is a true copy of publication 91.

1.17. In addition to the above research we have been isolating genes involved in angiogenesis with the aims of expanding our understanding of the downstream targets of endothelial cell activation and thus ultimately defining novel therapeutic targets. That research continues today.

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure JRG-3** referred to in my Statutory Declaration made this Twelth day of December 2000.

Jennifer Ruth Gamble

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12 December 2000/mb.-

TIMBRE CANTONAL Fr 2.50

CHRISTEN NOTAIRE à GENÈVE

WITNESS: ______________________

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

DOCUMENT LIST

Documents provided to me by the Patent Attorneys representing HGS in the subject proceedings are as follows:

1. U.S. Patent No. 6,130,071, issued October 10, 2000, to Alitalo et al.